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(54) **FERTILIZER/PLANT VITALIZER**

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- **TETUO TOMIYAMA: "THE SOLUBILITIES OF /-PROLINE AND /-HYDROXYPROLINE IN WATER, THE CALCULATED HEATS OF SOLUTION, AND THE PARTIAL MOLAL VOLUME OF /-HYDROXYPROLINE", THE JOURNAL OF GENERAL PHYSIOLOGY, 20 November 1935 (1935-11-20), pages 379-382, XP55032531,**
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- **SCHOBERT B ET AL: "Unusual solution properties of proline and its interaction with proteins", BIOCHIMICA ET BIOPHYSICA ACTA (BBA) - GENERAL SUBJECTS, ELSEVIER, AMSTERDAM, NL, vol. 541, no. 2, 15 June 1978 (1978-06-15), pages 270-277, XP023495169, ISSN: 0304-4165, DOI: 10.1016/0304-4165(78)90400-2 [retrieved on 1978-06-15]**

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**Description**

Technical Field use as

- 5 **[0001]** The present invention relates to a composition for use as a fertilizer or a plant vitalizer, which is composed of an aqueous solution of L-proline (hereinafter, sometimes simply referred to as "proline").

Background Art

- 10 **[0002]** Proline which is a kind of amino acids is known to have effects as a fertilizer or a plant vitalizer, and there are many prior literatures describing the effects thereof, such as Patent Document 1. Products advertising these effects are being sold.

- 15 **[0003]** To date, the proline has been available in a large number of product forms as a raw material for pharmaceuticals or foods. However, the proline in any form is in a crystalline or a powdery state, so proline is preserved, distributed, and sold in solid product forms with almost no moisture content. Among these products, Hanakagami (trade name) manufactured by and sold from Shoko Co., Ltd. is known as a product which contains a high percentage of proline and which is currently sold in Japan as a fertilizer or a plant vitalizer. Similarly, Hanakagami is preserved, distributed, and sold in a solid powdery state.

- 20 **[0004]** Proline products which are preserved, distributed, and sold as solids suffer from caking and deliquescence, due to the high hygroscopic property of proline, which greatly deteriorates the quality of the products. In order to avoid these problems, and to prevent moisture absorption by proline, conventional solid commercial products are required to be enclosed with silica gel having a hygroscopic property stronger than that of the proline, or be packed in a packaging material such as an expensive aluminized film which has almost no moisture permeability.

- 25 **[0005]** The main reason proline is distributed as a solid despite the cumbersome burden and cost as described above is that liquid proline solution easily decomposes and rots from exposure to microorganisms. In other words, many amino acids, including proline, when in aqueous solution without an additive which prevents microbial contamination, such as a preservative or a microbicide, start to gradually decompose due to microbial contamination within several days because of their high nutritive value. This effect is even seen when proline is in a normal preservation environment, but not an aseptic environment. If these problems did not exist, liquid proline in a liquid state is preferably distributed for application as a fertilizer or a plant vitalizer, because it is excellent in preservability, operability, and a distribution property.

- 30 **[0006]** Accordingly, there are only a few known documents that describe forms of preservation and distribution, and a distribution method for a proline product for a fertilizer or a plant vitalizer, particularly a proline product in a liquid state. When descriptions are made of these, there is always described that an additive such as a preservative or a microbicide is added to a liquid containing proline in order to prevent microbial contamination.

- 35 **[0007]** For instance, Patent Document 2 describes that a hydrolysate of proline or of a protein containing proline, or an amino acid mixture, is used as the source of proline for a flower-bud formation accelerator which contains a combination of proline and uracil. However, a liquid is not referred to as the form of the raw material proline to be used upon preparation of the flower-bud formation accelerator.

- 40 **[0008]** Meanwhile, Patent Document 3 describes, as a solution containing various amino acids including proline, a solution obtained by decomposing proteins of soybean, a cereal grain, or a microbial cell body, by various methods, and fermentation solutions of various amino acids. However, there is no description about the form of preservation, the form of distribution, and the distribution method of the solution containing amino acids including proline.

- 45 **[0009]** In addition, as examples of application of the proline to a plant, Patent Documents 4 to 6 describe an amino acid fermentation solution containing proline, alanine, valine, glutamic acid, and the like, which has been subjected to the removal of cell bodies or sterilization. However, there is no specific description about the form of preservation, the form of distribution, and the distribution method of such the amino acid-containing solution.

- 50 **[0010]** Patent Document 7 describes that an amino acid fermentation solution to be applied to a plant may be in a form of a mixture with an organic or inorganic material which is blended in order to facilitate the storage, transport, or handling of the amino acid fermentation solution. That is, it is described that, in order to cover the shortcomings of the amino acid fermentation solution, an organic material or inorganic material of any appropriate kind for improving the preservability or the ability to be distributed is preferably added. Specific examples of these material to be added for improving the storage property include, when it is a liquid, various alcohols, ethers, ketones, aromatic hydrocarbons, paraffinic hydrocarbons, and aldehydes.

- 55 **[0011]** Patent Document 7 describes that a solution, which is obtained by removing cell bodies from an amino acid fermentation solution containing various amino acids. This solution undergoes a change in the components due to the proliferation of contaminated microorganisms if nothing is done for the solution, except when a case where the solution is used immediately. Therefore, it is preferable that the solution be preserved after the pH thereof is adjusted to 3 or less to stabilize the quality. In general, a large amount of a strong mineral acid such as hydrochloric acid or sulfuric acid

has to be added in order to adjust the pH of the resulting fermentation solution so that it has a pH around neutral to 3 or less.

**[0012]** Patent Document 9 describes that an additive such as a surfactant, a pH regulator, or a preservative may be added to an amino acid fermentation solution, as required. Patent Document 10 describes the form of a proline-containing product and also describes that the proline-containing product which is prepared with an addition of a surfactant, when used in a liquid form. Furthermore, Patent Document 11 describes that proline-containing products can be blended with a microbicide, a surfactant, or a preservative from the viewpoint of prevention from spoilage by microbes.

Patent Document 1:JP-A 2001-131009

Patent Document 2:JP-B 46-42566

Patent Document 3:JP-B 56-32961

Patent Document 4:Japanese Patent No. 2852677

Patent Document 5:Japanese Patent No. 2874788

Patent Document 6:Japanese Patent No. 2874789

Patent Document 7:JP-A 06-80530

Patent Document 8:Japanese Patent No. 3377873

Patent Document 9:JP-A 2001-192310

Patent Document 10:JP-A 2002-199812

Patent Document 11:JP-A 2003-48803

**[0013]** Tetuo Tomayama et al., The Journal of General Physiology, 20 November 1935, pages 379-382, concerns the solubilities of L-proline and L-hydroxyproline in water, the calculated heats of solution, and the partial molar volume of L-hydroxyproline.

**[0014]** JP 63 045211 A concerns a method for increasing the yield of rice, fruit and vegetable, edible root, flower and ornamental plant, fruit tree, or the like.

**[0015]** JP 57 002691 A concerns preparation of L-proline by fermentation method.

**[0016]** JP 59 066893 concerns preparation of L-proline.

#### Disclosure of the Invention

**[0017]** The conventionally known problems associated with proline-containing solutions are caused by the use of various additives for suppressing proliferation of microorganisms in order to prevent deterioration in preservability of the solution caused by microbial contamination.

**[0018]** In general, fertilizers or a plant vitalizers containing proline are applied by mixing or injecting these compositions into cropland soil on which plants are growing, or dispersing onto the parts of the plants which are above ground. Therefore, when a fertilizer or a plant vitalizer containing proline is applied to a plant, the various additives added to the proline-containing solution are concomitantly applied not only to the plants, but also to the surrounding areas. As a result, for example, when a preservative, a microbicide, or the like is applied to field crops, an adverse effect is seen on the growth of various organisms including soil bacteria in the soil of the farmland. In recent years, various agricultural chemicals and environmental hormones left in the soil have become problematic, and prevention of environmental pollution which accompanies agricultural production is a great social concern.

**[0019]** Furthermore, also when a preservative or a bactericide as described above is applied in a facility such as a greenhouse, the influence on the surrounding environment has certainly become problematic, and the adverse effects on the health of workers resulted in even more problem.

**[0020]** In addition, a method which involves adding an acid to the proline-containing solution to lower the pH of the solution and thereby suppress the proliferation of contaminated microorganisms includes also problems related to the environmental destruction, similar to the problem with the addition of a bactericide. This is because the acidic solution is dispersed around the plant when the proline-containing solution is applied to the plant.

**[0021]** Furthermore, use of a proline-containing solution in which the pH thereof is decreased by adding an acid, particularly a mineral acid such as hydrochloric acid or sulfuric acid, causes the accumulation of inorganic ions of hydrochloride and sulfide in the soil on which the plant applied with the proline-containing solution grows, leading to soil pollution which is called salt accumulation. There is a concern that repeated application of the proline-containing solution for many years may cause a so-called salt damage, resulting in inhibition of the growth of plants due to an increase in the salt concentration of the cropland soil.

**[0022]** The problems accompanied with those bacteriocides, preservatives, and acidic solutions are that the repeated applications of those gradually cause environmental pollution, even if the proline-containing solution as a fertilizer or a plant vitalizer is used after being diluted with a large amount of water, so the essential problems are not solved.

**[0023]** Thus, an object of the present invention is to provide a proline solution to be used as a fertilizer or a plant vitalizer, which has improved preservability, operability, and distribution without using additives which may cause the

environmental pollution as described above.

**[0024]** The inventors of the present invention have extensively studied to achieve the above-mentioned object. As a result, they found that a proline-containing solution exhibits a significant effect of preventing spoilage due to microbes without impairing the operability of the solution by adjusting a proline concentration in the solution within an appropriate range, and thus have completed the present invention on the basis of such the observation.

**[0025]** That is, the present invention is as described hereinbelow.

1. Use of an aqueous L-proline solution having an L-proline concentration of 20% (w/w) to 80% (w/w) as a fertilizer or a plant vitalizer, wherein the aqueous L-proline solution is obtainable by culturing a microorganism having L-proline-producing ability in a medium, producing and accumulating L-proline in the medium, removing cell bodies from a resulting L-proline fermentation solution, desalting the L-proline fermentation solution, and concentrating the solution.

2. Use of composition according to item 1, wherein the L-proline concentration is 40 to 70% (w/w).

#### Best Mode for carrying out the Invention

**[0026]** Hereinafter, the present invention will be described in detail. Note that, herein, the term "proline" herein refers to "L-proline".

**[0027]** The composition to be used as a fertilizer or a plant vitalizer described herein is for improving the growth of a plant by being applied to the plant. Typical examples of the plant of interest include: flowers and ornamental plants such as rose and pansies; vegetables such as cucumbers, tomatoes, strawberries, melons, and spinach; fruits such as pears, mandarin oranges, and grapes; and cereal grains such as rice and beans. Meanwhile, examples of the application method include mixing or injecting into the cropland soil where the plants are growing, and dispersion onto the above-ground parts of the plants.

**[0028]** The composition for a fertilizer or a plant vitalizer described herein is composed of an aqueous L-proline solution having an L-proline concentration of 20% (w/w) or more.

**[0029]** In general, when an aqueous solution of an amino acid is subjected, for example, to vaporization of the moisture in the aqueous solution for the purpose of adjusting the amino acid concentration or the moisture content, the moisture content in the aqueous solution decreases while the amino acid concentration increases, resulting in an increase in the osmotic pressure of the aqueous solution. As a consequence, many microorganisms cannot survive such an environment in which the osmotic pressure exceeds a level at which the bacteria can survive in the solution. However, the extent to which the moisture content is decreased and the osmotic pressure is increased in order to suppress the proliferation of the microorganisms such as saprophytic microorganisms depends on the kind of solution, the kind of solute, physical conditions such as temperature or pH, and the like, and thus is not constant.

**[0030]** On the other hand, the amino acid concentration in the aqueous solution gradually increases as the moisture evaporates, and the dissolved amino acids become crystals and precipitate when the concentration exceeds saturation of the amino acids with respect to water. The amino acid aqueous solution in which the crystal is precipitated cannot maintain its uniformity because the crystal deposits on a bottom portion, and has an inferior operability because the solid component is mixed therein and thus is not preferable for preservation or for distribution. In addition, if the moisture content is further decreased when the crystal precipitates, the amino acid concentration of the solution does not increase. Therefore, if the osmotic pressure at that time is within a range in which a bacterium can proliferate, the effect of preventing spoilage of the solution by microbes by further adjustment of the moisture content will most likely not occur.

**[0031]** Accordingly, to date, the preservation condition, distribution condition, and distribution method, in which such methods of adjusting the moisture content in an amino acid aqueous solution are used to prevent the decomposition of the amino acids by a bacterium, have hardly been put to practical use. In particular, such distribution of proline have not been previously disclosed.

**[0032]** The inventors of the present invention have investigated relationships between the proline concentration in an aqueous proline solution, the growth of microorganisms in the solution, and the operability of the solution. As a result, they have found that the proline concentration of a solution can be adjusted to within a particular range, which makes it possible to prevent the proliferation of microorganisms without impairing the operability of the solution. In addition, the packing of such products is made compact by increasing the proline concentration, thereby making it possible to improve the distribution in view of its of preservation, transport, and the like.

**[0033]** The proline concentration of the solution described herein is preferably 80% (w/w) or less, more preferably 20 to 70% (w/w), and still more preferably 40 to 70% (w/w). A particularly preferable proline concentration is 50% (w/w). The proline concentration is adjusted within the above-mentioned range, thereby making it possible to prevent the proliferation of contaminated microorganisms. Meanwhile, when the proline concentration is within this range, the precipitation of proline during preservation can be prevented, and the viscosity of the proline solution does not become too

high. Therefore, physical properties suitable for distributing the solution using a pump or spraying the solution can be maintained.

**[0034]** The proline solution described herein can be produced by, for example, dissolving proline in water such that the proline concentration is within the above-mentioned range. The proline solution described herein may contain components other than proline as long as the effectiveness of the solution is not impaired. Examples of the other components include other fertilizer components, plant vitalizers, plant growth regulators, vitamins, minerals, spreaders, and other generally applied agricultural/horticultural materials. However, when the proline solution described herein is used as a fertilizer or a plant vitalizer and dispersed onto cropland, the amount of non-proline components is preferably small so to prevent the growth inhibition (e.g., salt damage) of the plants and resulting environmental pollution.

**[0035]** The term "solution" or "aqueous solution" in the present invention means that a solvent is water or a water-based solution. The water-based solution may contain a water-soluble organic solvent such as an alcohol such as ethanol. The concentration of the water-soluble organic solvent is not particularly limited as long as a certain solubility of proline can be stably maintained. For instance, when the solvent includes ethanol, the concentration thereof is preferably 20% (w/w) or less and preferably 10% (w/w) or less.

**[0036]** On the other hand, from an economic viewpoint, a more inexpensive raw material may preferably be used as long as the effectiveness of the proline solution is not impaired. An example of such a raw material includes a proline fermentation solution obtained by using a microorganism having an ability to produce proline.

**[0037]** When a proline fermentation solution is used as the raw material for the proline solution described herein, the method of adjusting the proline concentration is not particularly limited. For instance, microbial cell bodies in the proline fermentation solution are removed, and then the proline concentration can be adjusted by using a device for removing a moisture which is generally known such as a vacuum evaporator or a reverse osmosis membrane. The proline fermentation solution in which the cell bodies have been removed can be supplemented with solid proline such as a proline crystal or a liquid containing a high concentration of proline, to increase the proline concentration. This increases the proline concentration in the fermentation solution without the cell bodies. In the present invention, salts, except proline, are preferably removed from the proline fermentation solution from which cell bodies have been removed. Examples of such salts include inorganic salts which are derived from the medium. Examples of the desalting method include an ion-exchange resin treatment, electroosmosis, and a reverse osmosis membrane treatment. The ratio (i.e., weight ratio) of contaminating salts to proline in the proline solution is preferably 1 or less, more preferably 0.5 or less, or still more preferably 0.3 or less.

**[0038]** The proline fermentation solution can be obtained by culturing a microorganism which has an ability to produce L-proline in a medium so that L-proline is produced and accumulates in the medium. The above-mentioned microorganism is not particularly limited as long as it has the ability to produce proline, and examples thereof include bacteria belonging to the genus *Escherichia*, coryneform bacteria, and bacteria belonging to the genus *Serratia*. Specific examples thereof include the following strains, but the present invention is not limited to these strains.

*Escherichia coli* AJ11543 (FERM P-5483) (JP-A 56-144093)  
*Escherichia coli* AJ11544 (FERM P-5484) (JP-A 56-144093)  
*Brevibacterium lactofermentum* AJ11225 (FERM P-4370) (JP-A 60-87788)  
*Brevibacterium flavum* AJ11512 (FERM P-5332) (JP-B 62-36679)  
*Brevibacterium flavum* AJ11513 (FERM P-5333) (JP-B 62-36679)  
*Brevibacterium flavum* AJ11514 (FERM P-5334) (JP-B 62-36679)  
*Corynebacterium glutamicum* AJ11522 (FERM P-5342) (JP-B 36679)  
*Corynebacterium glutamicum* AJ11523 (FERM P-5343) (JP-B 62-36679)

**[0039]** The microorganism which can be used in the proline fermentation may be a wild-type strain, or may be a mutant or a recombinant strain obtained by breeding a microorganism to improve the ability to produce proline.

**[0040]** The medium which can be used in the proline fermentation may be one which is conventionally used in proline fermentation, and which contains a carbon source, a nitrogen source, inorganic ions, and as required, organic micronutrients such as amino acids and vitamins.

**[0041]** Examples of the carbon source include saccharides such as glucose, fructose, sucrose, and maltose; saccharified starch solutions containing these saccharides; sweet potato molasses, beet sugar molasses, high test molasses, organic acids such as acetic acid, and alcohols such as ethanol and glycerine.

**[0042]** Examples of the nitrogen source include nitrogen-containing raw materials such as ammonia gas, ammonia water, ammonium salts, urea, and nitric acid.

**[0043]** The culture conditions are not particularly different from the conventional method of culturing a proline-producing bacterium.

**[0044]** The proline solution described herein can be preserved and distributed without being contaminated by microorganisms, by adjusting the proline concentration within the above-mentioned range. Examples of the contaminated

microorganism include microorganisms belonging to the genus *Bacillus*, *Aspergillus*, or *Saccharomyces*.

#### Examples

[0045] Hereinafter, the present invention will be described in more detail by referring to examples.

[Reference example 1] Production of the proline fermentation solution

#### (1) Proline fermentation

[0046] A proline fermentation solution can be obtained by a method described in Example 2 in JP-A 05-284985. Specifically, it can be produced in a manner as described below.

[0047] A liquid medium having the composition shown in Table 1 was prepared and adjusted to a pH of 7.2. Then, 20 ml of the liquid medium was added to a 500-ml shaking flask and heat-sterilized. One platinum loop of cells of *Corynebacterium glutamicum* AJ11522 which previously had been grown in a natural medium was inoculated into the liquid medium, and was cultured with shaking at 30°C for 72 hours. After completion of the culture, typically, 3.4 g/dl of L-proline is present in the culture solution.

[0048] The AJ11522 strain is L-isoleucine auxotrophic, and is an L-proline-producing bacterium which has a high citrate synthetase activity (JP-A 05-284985).

Table 1

| Component                                       | Concentration |
|-------------------------------------------------|---------------|
| Glucose                                         | 10 g/dl       |
| KH <sub>2</sub> PO <sub>4</sub>                 | 0.1 g/dl      |
| (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> | 6.0 g/dl      |
| MgSO <sub>4</sub> ·7H <sub>2</sub> O            | 0.04 g/dl     |
| FeSO <sub>4</sub> ·7H <sub>2</sub> O            | 1 mg/dl       |
| MnSO <sub>4</sub> ·4H <sub>2</sub> O            | 1 mg/dl       |
| Biotin                                          | 250 ug/l      |
| Thiamine hydrochloride                          | 500 ug/l      |
| Hydrolysate of soybean protein                  | 0.3 ml/dl     |
| Isoleucine                                      | 15 ml/dl      |
| CaCO <sub>3</sub> (separately sterilized)       | 5 g/dl        |

[0049] Next, the cell bodies were removed from the culture solution using a centrifuge. Then, liquid was evaporated from the solution by heat under vacuum using a rotary evaporator, to thereby obtain 1.2 ml of a concentrated solution. The proline concentration in the concentrated solution was about 50 g/dl.

#### (2) Production of the desalted, concentrated proline solution

[0050] The method of desalting the proline fermentation solution can be performed according to a conventional method. Hereinafter, a method employing an ion-exchange resin is shown as an example.

[0051] The proline fermentation was performed by a method described in the above-mentioned section (1) or any other appropriate method. Sulfuric acid was added to 320 ml of the resultant proline fermentation solution (e.g., a solution containing 20 g of proline) until the pH was 3. Then, the cell bodies were removed by ultrafiltration. 450 ml of the resulting proline solution was passed through 100 ml of a commercially available cationic ion-exchange resin (H type) having strong acidity, which had been loaded onto a column. Proline in the solution is allowed to adsorb to the resin, thereby separating proline from the contaminating anions present in the fermentation solution. Then, an eluting solution of 200 ml of 1 N NaOH was passed through the column, followed by water (typically, about 600 ml) until the proline, including proline which had adsorbed to the resin, is completely eluted from the column. 700 ml of an eluate obtained by performing the treatment as described above the proline fermentation solution which had been obtained by the method described in the above-mentioned section (1) or any other appropriate proline fermentation contained about 16 g of proline in a concentration of about 2.3 g/dl. Furthermore, the desalted solution can be concentrated by heat under vacuum to remove the moisture, to thereby obtain, for example, 32 ml of a proline solution. The proline concentration of the solution was 50 g/dl.

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[Example 1] Proline concentration in an aqueous proline solution and the proliferation of microorganisms

**[0052]** The proliferation of microorganisms in aqueous proline solutions at various concentrations was investigated according to the method as described below.

**[0053]** Proline crystals (manufactured by Ajinomoto Co., Inc.) having a quality of a commercially available raw material for pharmaceutical preparations were dissolved in tap water to obtain proline solutions at the concentrations shown in Tables 2 and 3, to thereby obtain 50 ml of each aqueous solutions. Then, 0.5g of unsterilized soil was added to each proline solution to add microorganisms, and the mixtures were preserved in bottles at 25°C and 40°C for 4 weeks. Tables 2 and 3 show the proliferation of microorganisms in each proline solution after the preservation.

Table 2. Proliferation of microorganisms in aqueous proline solution (preserved at 25°C for 4 weeks)

| Proline concentration (%)                        | Number of days for preservation |   |   |    |    | pH of solution on the 28th day | Osmotic pressure (Osm/kg) | Operability of solution |
|--------------------------------------------------|---------------------------------|---|---|----|----|--------------------------------|---------------------------|-------------------------|
|                                                  | 0                               | 3 | 7 | 14 | 28 |                                |                           |                         |
| 0                                                | -                               | - | - | -  | -  | 6.6                            | 0                         | Good                    |
| 10                                               | -                               | + | + | +  | +  | 6.4                            | 0.9                       | Good                    |
| 20                                               | -                               | - | + | +  | +  | 6.5                            | 1.8                       | Good                    |
| 30                                               | -                               | - | + | +  | +  | 6.5                            | 2.7                       | Good                    |
| 40                                               | -                               | - | - | +  | +  | 6.6                            | 3.6                       | Good                    |
| 50                                               | -                               | - | - | -  | -  | 6.9                            | 4.5                       | Good                    |
| -: no appearance of fungi +: appearance of fungi |                                 |   |   |    |    |                                |                           |                         |

Table 3. Microorganism proliferation in aqueous proline solution (preserved at 40°C for 4 weeks)

| Proline concentration (%)                        | Number of days for preservation |   |   |    |    | pH of solution in the 28th day | Osmotic pressure (Osm/kg) | Operability of solution |
|--------------------------------------------------|---------------------------------|---|---|----|----|--------------------------------|---------------------------|-------------------------|
|                                                  | 0                               | 3 | 7 | 14 | 28 |                                |                           |                         |
| 0                                                | -                               | - | - | -  | -  | 6.5                            | 0                         | Good                    |
| 10                                               | -                               | + | + | +  | +  | 6.6                            | 0.9                       | Good                    |
| 20                                               | -                               | - | + | +  | +  | 6.6                            | 1.8                       | Good                    |
| 30                                               | -                               | - | + | +  | +  | 6.6                            | 2.7                       | Good                    |
| 40                                               | -                               | - | - | +  | +  | 6.6                            | 3.6                       | Good                    |
| 50                                               | -                               | - | - | -  | -  | 6.9                            | 4.5                       | Good                    |
| -: no appearance of fungi +: appearance of fungi |                                 |   |   |    |    |                                |                           |                         |

**[0054]** As shown above, it was confirmed that, when the proline concentration in an aqueous solution was 10% or more, the proliferation of microorganisms was suppressed. In addition, it was confirmed that, the proliferation of the microorganisms was further suppressed as the proline concentration increased, and when the proline concentration was about 50%, the proliferation of the microorganisms was prevented almost completely.

**[0055]** It should be noted that values of the pH of the aqueous proline solutions after four weeks were almost uniform, indicating that the proliferation of the microorganisms was not suppressed by the change in pH.

**[0056]** In addition, every solution had good operability, and no precipitation of crystals.

[Example 2] Concentration of contaminating salts in proline solution and the consequent salt damage to plant

**[0057]** The effects of contaminating salts in a proline solution and its relationship to salt damage was investigated using model solutions. For the model solutions, a solution obtained by removing cell bodies from a proline fermentation

solution (hereinafter, simply referred to as the "proline fermentation solution") and a solution obtained by removing the contaminating salts from the fermentation solution (hereinafter, referred to as the "desalted solution") were used. Table 4 shows the composition of the proline fermentation solution, and Table 5 shows the composition of the desalted solution.

**[0058]** Komatsuna (i.e., *Brassica campestris* var. *peruviridis*) was cultivated in a pot (having a diameter of 7.5 cm and a depth of 6.5 cm) with a commercially available culture soil. 2.5 ml of each model solution was added once onto the surface of leaves of komatsuna, to confirm the degree of a salt damage. Table 6 shows results obtained 18 hours after the dispersion.

Table 4. (units in Table are weight%)

|                                                 | A       | B        |
|-------------------------------------------------|---------|----------|
| Proline                                         | 0.02    | 0.002    |
| KH <sub>2</sub> PO <sub>4</sub>                 | 0.00056 | 0.000056 |
| (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> | 0.03428 | 0.003428 |
| MgSO <sub>4</sub> ·7H <sub>2</sub> O            | 0.00024 | 0.000024 |
| NaCl                                            | 0.00172 | 0.000172 |
| Water                                           | 99.94   | 99.99    |
| Total inorganic salt concentration              | 0.0368  | 0.00368  |

Table 5. (units in Table are weight%)

|         | C      | D      | E      |
|---------|--------|--------|--------|
| Proline | 0.020  | 0.002  | 0.000  |
| Water   | 99.980 | 99.998 | 100.00 |

Table 6

|                                                                                                                                                                                                         | A        | B        | C    | D    | E      |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------|----------|------|------|--------|
| Growth                                                                                                                                                                                                  | Not good | Not good | Good | Good | Normal |
| "Not good" indicates the withering of leaves or stems, i.e. unvigorous growth, as compared to Control Plot E "Good" indicates vigorous leaves or stems, i.e. good growth, as compared to Control Plot E |          |          |      |      |        |

**[0059]** As described above, the salt damage to a plant was suppressed by decreasing the concentration of contaminating salts at any proline concentration. Also, higher concentration proline solutions were able to be dispersed. In addition, it was confirmed that the dispersion of a high concentration proline solution improves the growth of a plant.

#### Industrial Applicability

**[0060]** The composition for a fertilizer or a plant vitalizer described herein has excellent operability because it is a liquid, can prevent contamination by microorganisms, and in addition, likely do not cause a salt damage to a plant when applied thereto.

#### Claims

1. Use of an aqueous L-proline solution having an L-proline concentration of 20% (w/w) to 80% (w/w) as a fertilizer or a plant vitalizer,  
wherein the aqueous L-proline solution is obtainable by culturing a microorganism having L-proline-producing ability in a medium, producing and accumulating L-proline in the medium, removing cell bodies from a resulting L-proline fermentation solution, desalting the L-proline fermentation solution, and concentrating the solution.

2. Use of composition according to claim 1, wherein the L-proline concentration is 40 to 70% (w/w).

## Patentansprüche

1. Verwendung einer wässrigen L-Prolin-Lösung mit einer L-Prolin-Konzentration von 20% (w/w) bis 80% (w/w) als Düngemittel oder Pflanzen-Vitalisierungsmittel (plant vitalizer), wobei die wässrige L-Prolin-Lösung erhältlich ist durch Kultivieren eines Mikroorganismus mit L-Prolin-Produktionsfähigkeit in einem Medium, Produzieren und Akkumulieren von L-Prolin in dem Medium, Entfernen von Zellkörpern aus einer resultierenden L-Prolin-Fermentationslösung, Entsalzen der L-Prolin-Fermentationslösung und Konzentrieren der Lösung.
2. Verwendung einer Zusammensetzung gemäß Anspruch 1, wobei die L-Prolin-Konzentration 40 bis 70% (w/w) beträgt.

## Revendications

1. Utilisation d'une solution aqueuse de L-proline ayant une concentration en L-proline de 20% (poids/poids) à 80% (poids/poids) comme fertilisant ou vitalisant de plante, dans laquelle la solution aqueuse de L-proline peut être obtenue en cultivant un micro-organisme ayant une capacité de production de L-proline dans un milieu, en produisant et en accumulant la L-proline dans le milieu, en éliminant les corps cellulaires provenant d'une solution de fermentation de L-proline résultante, en désalinisant la solution de fermentation de L-proline et en concentrant la solution.
2. Utilisation de la composition selon la revendication 1, dans laquelle la concentration en L-proline est de 40 à 70 % (poids/poids).

## REFERENCES CITED IN THE DESCRIPTION

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